EVALUATION OF PASSIVE IMMUNITY TO SCHISTOSOMIASIS IN MICE TRANSFUSED WITH HYPER-IMMUNE BABOON SERUM

PATRICK KIARIE NDING’URI (B.Sc)
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NOVEMBER 2009
DECLARATION

I Patrick Kiarie Nd’uri, duly declare that this thesis is my original work and has not been presented for a degree in any other University or any other award.

Patrick Kiarie Nd’uri

Signature: …………………..Date:…………………………………………

Supervisors Approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as the University supervisors.

Dr. Joseph N. Makumi
Department of Biochemistry and Biotechnology
Kenyatta University

Signature……………………..Date…………………………………………

Dr. Joseph J N. Ngeranwa
Department of Biochemistry and Biotechnology
Kenyatta University

Signature……………………..Date…………………………………………

Dr. Thomas Kariuki
Department of Tropical and Infectious Diseases
Institute of Primate Research

Signature……………………..Date…………………………………………
DEDICATION

This thesis is dedicated to my dear parents: Mr. Stephen Nding’uri and Mrs. Mary Wangui Nding’uri. You are the pillars of my life.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DALY</td>
<td>Disability adjusted life years</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>IFN-λ</td>
<td>Interferon-gamma</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IPR</td>
<td>Institute of Primate Research</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>RA</td>
<td>Radiation attenuated</td>
</tr>
<tr>
<td>SEA</td>
<td>Soluble egg antigen</td>
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<tr>
<td>SWAP</td>
<td>Soluble adult worm preparation</td>
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<tr>
<td>Th</td>
<td>T helper</td>
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<td>KLH</td>
<td>Keyhole Limpet Hemocyanin</td>
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ABSTRACT

Schistosomiasis is a tropical disease caused by worms in the genus Schistosoma. It remains one of the most prevalent parasitic infections and has significant economic and public health consequences. Despite having an effective chemotherapeutic drug, praziquantel, there is still need for a vaccine since the immediate manifestations in most people are negligible or indeterminate. Several antigens have been tested as vaccine candidates with largely disappointing results. Both rodents and primates exposed to cercariae larvae optimally attenuated with gamma radiation show a highly significant reduction in challenge worm burden. Vaccination of baboons with RA (Radiation attenuated) vaccine gave 54% protection after three exposures and 86% protection after five exposures. Protection due to RA vaccine is both humoral and cellular mediated with the IgG antibody levels thought to mediate the effector response declining over a relatively short period after the last vaccination. This study therefore aimed at evaluating the role of humoral immunity against migrating and or the pre-liver lung stage schistosomulæ by passively immunizing mice with either 500 µl of hyper-immune serum or infection serum from baboons. In the first study, mice were immunized on day 7 post challenge and boosted on the 14th day. In the second study, mice were immunized on the 3rd day post challenge with no boost. A 24 hour egg count was carried out on the 5th, 6th, and 7th week post challenge using Kato Katz thick smear technique after which perfusion was done seven weeks post challenge. Blood was collected at week 0, 2, and 4 in experiment two from the tail and through heart puncture during perfusion in both experiments for IgG ELISA assays. Sections of preserved livers were processed for histopathology and for eggs recovery. Immunization against the migrating schistosomes on the 3rd day with the hyper-immune serum gave a 25.8% protection but there was no protection obtained when pre-liver lung stage schistosomulæ was targeted. There was no significant difference between the 24 hour faecal egg number, number of worms, liver eggs and in number and size of granulomas on both experiment. There was thus no significant protection that was conferred in mice against pre-liver-lung stage schistosomulæ and or the migrating schistosome larvae by hyper-immune serum from RA vaccinated baboons probably due to the inability of the baboon serum to cooperate with the mouse effector system necessary in killing the schistosomulæ. These results suggest that protective mechanism in baboons against schistosome worms could be different from those in mice and that though antibodies could be involved in protection, other factors such as cytokines could also play a major role. There is thus the need to further evaluate the mechanisms of protection in mice and experiment on homologous passive transfer of hyper-immune serum (baboon to baboon). Other stages of the schistosome lifecycle should also be targeted during passive transfer experiments.
CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Schistosomiasis commonly known as bilharzia is a tropical parasitic disease caused by blood dwelling flukes of the genus *Schistosoma*. It is the most significant helminthic infection in humans because of its global prevalence, nature of its associated disease manifestations and the remarkable difficulties encountered in attempts to control its spread. Five species of *Schistosoma* are known to infect humans; *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium*, and *S. intercalatum*. Of these, three species account for most of the human infection: *Schistosoma mansoni* that mainly causes hepatic and intestinal schistosomiasis, *S. haematobium* which causes the urinary form of the disease, and *S. japonicum* that results to hepatosplenic and intestinal schistosomiasis (Chitsulo et al., 2000). The disease has been ranked second only to malaria and is one of the 10 tropical diseases that are targeted for control by the Special Program for Research and Training in Tropical Diseases of the United Nations Development Program, the World Bank, and the World Health Organization (Morel, 2000).

1.1.1 Geographic Distribution and Disease burden

Schistosomiasis being a water-borne disease that is transmitted by freshwater snails is endemic in 76 countries of the tropics and sub-tropics afflicting populations that live close to the water reservoirs (lakes, ponds, and irrigation canals) that are contaminated with the parasite (Chitsulo et al., 2000). WHO (2002) estimated that 200 million people are infected and 779 million at risk of infection with 85% of the latter living in Africa.
alone (Steinmann et al., 2006). Of the estimated 200 million people that are infected, approximately 80% of them are in sub-Saharan Africa (WHO, 2002).

The distribution of the different species of schistosomes mainly depends on the ecology of the snail hosts. Natural streams, ponds, and lakes are typical sources of infection, but over the past few decades man-made reservoirs and irrigation systems have contributed to the spread of schistosomiasis (Oomen et al., 1990). Snail populations, cercarial density, and patterns of human water contact show strong temporal and spatial variations thus resulting in a focal distribution of the infection within countries, regions, and villages (Gryseels and Nkulikyinka, 1998). It has been observed that within a population and age-groups, schistosomes are over-dispersed thus implying that a small number of individuals carry most of the parasites (Gryseels and De Vlas, 1996). This could be attributed both to water-contact patterns as well as to innate and acquired immunity. Also observed to vary within a population is the sex-related patterns in relation to behavioural, professional, cultural, and religious factors (Jordan et al., 1993). Though the disease is largely a rural problem, urban foci can be found in many endemic areas (Mott et al., 1990).

Despite the high prevalence, morbidity associated with the disease is low and variable. Currently, the Global Burden of Disease Study attributes a disability weight of 0.06 and an annual mortality of 14,000 deaths per year due to schistosomiasis. The total number of DALY (disability-adjusted life years) lost to schistosomiasis is estimated at 1.532 million per year, of which 77% are in sub-Saharan Africa (Lopez, et al., 2006). Schistosomiasis thus accounts for 0.1% of the total world global burden of disease and
0.4% of that in sub-Saharan Africa, which is of the same order as leishmaniasis and trypanosomiasis (Chitsulo et al., 2000).

Despite major advances in control and substantial decreases in morbidity and mortality, schistosomiasis continues to spread to new geographic areas. This spread has been shown to be facilitated by both the environmental changes that result from the development of water resources as well as the growth and migration of populations (Patz et al., 2000). A good example is the construction of Diama Dam on the Senegal River which led to the introduction of S. mansoni into Mauritania and Senegal. The movement of refugees and the displacement of populations resulted in the introduction of S. mansoni into Somalia and Djibouti. The presence of the Aswan Dam in Egypt has also led to the virtual elimination of S. haematobium from the Nile Delta but has brought about the establishment of S. mansoni in upper Egypt (Ross et al., 2001).

1.1.2 Biology and life cycle of the parasite

The main schistosomes infecting human beings are: S. mansoni, which is transmitted by Biomphalaria snails; S. haematobium, transmitted by Bulinus snails; and S. japonicum, transmitted by the amphibian snail Oncomelania. S. intercalatum and S. mekongi are of less importance while S. japonicum is a zoonotic parasite which infects a wide range of animals including cattle, dogs, pigs, and rodents. S. mansoni is also found in rodents and primates, but human beings are the main host. The main intermediate snail hosts of S. mansoni are species of Biomphalaria (B) with some of these being B. pfeifferi, B. choanomphala, B. smithi, B. stanleyi, B.angulosa and B. sudanica in East Africa. Schistosomes have no second intermediate host in their life-cycle and thus mature in the
blood vascular system of their definitive hosts. Adult schistosomes are white or greyish worms of 7–20 mm in length with a cylindrical body that has two terminal suckers, a complex tegument, a blind intestinal tract, and reproductive organs. Unlike other trematodes, schistosomes have separate male and female sexes. The male's body has a groove or gynaecophoric canal, in which it holds the longer and thinner female. They feed on blood and globulins through anaerobic glycolysis and the released debris is regurgitated in the host's blood (Jordan, 1993).

Schistosomes undergo two stages of development that involves multiplicative asexual stage of development in the snail intermediate host and the sexual stage in vertebrates, the definitive host (Fig. 1.1). The asexual stage starts when the eggs of the schistosomes are passed by the definitive hosts (vertebrates) in urine for *S. haematobium* or faeces for *S. mansoni, S. japonicum, S. mekongi,* and *S. intercalatum* into the water (Jourdane and Theron, 1987). The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human-water contact. Under the guidance of light and chemical stimuli, these schistosome eggs from urine or faeces hatch in fresh water and release the *miracidium* (larva) that penetrates a snail of the appropriate species. The miracidia multiply asexually within the snail into multicellular sporocysts and later into cercarial larvae with embryonic suckers and a characteristic bifurcated tail. It is only after two generations — primary and then daughter sporocysts within the snail that cercariae are released (Jordan, 1993). Vast numbers of cercariae are released into the water 4-6 weeks after snail infection which through the secretion of cytolytic substances from the head glands can penetrate the definitive host where they
develop into schistosomula and eventually into adult worms (Jourdane and Theron, 1987).

The sexual stage occurs in vertebrates when they become infected with cercariae. Cercariae penetrate the skin of humans or, in the case of *S. japonicum*, humans and other
mammalian hosts that act as reservoirs for the infection. During infection, cercariae shed their bifurcated tails and the resulting schistosomula enter capillaries and lymphatic vessels and then move towards the lungs. After several days, the worms migrate to the portal venous system, where they mature and unite. Pairs of worms then migrate to the superior mesenteric veins (in the case of *S. mansoni*), the inferior mesenteric and superior hemorrhoidal veins (in the case of *S. japonicum*), or the vesical plexus and veins draining the ureters (in the case of *S. haematobium*) (Shiff, 2000). Egg production commences four to six weeks after infection and continues for the life of the worm — usually three to five years. Each ovum contains a ciliated miracidium larva, which secretes proteolytic enzymes that help the eggs to migrate into the lumen of the bladder (*S. haematobium*) or the intestine (other species). Eggs pass from the lumen of blood vessels into adjacent tissues, and most of them then pass through the intestinal or bladder mucosa and are shed in the feces (in the case of *S. mansoni* and *S. japonicum*) or urine (in the case of *S. haematobium*). The life cycle is completed when the eggs hatch releasing miracidia that, in turn, infect specific freshwater snails (Jourdane and Theron, 1987).

1.2 Disease patterns in man

1.2.1 Cercarial Dermatitis

This is the condition that manifest itself as a temporary urticarial rash soon after the percutaneous penetration of the cercariae. It can persist for days as papulopruriginous lesions especially after primary infection (Appleton, 1984). This condition is infrequent among those living in areas of endemicity, but migrants or visitors who are infected may develop it within a few hours of exposure. The resulting dermatitis is similar to
swimmer's itch, noted in persons sensitized and reexposed to avian or other nonhuman schistosomes found in freshwater bodies all over the world. It is not known if reexposure is a feature of cercarial dermatitis for *S. japonicum* (Warren, 1973).

1.2.2 Acute Schistosomiasis

Acute schistomiasis also known as Katayama fever is the systemic hypersensitivity reaction against the migrating schistosomulae occurring a few weeks to months after a primary infection (Lambertucci, 1993). It is common in areas of high transmission rates but seldom observed in endemic populations probably due to the early age (2-4 years) at which most of the children in the endemic areas are exposed to the parasite for the first time (Doherty, *et al*., 1996). Symptoms are thought to be mediated by the immune complex, and the majority of cases begin with the deposition of an egg into host tissues. Common symptoms include fever, headache, generalized myalgias, right-upper-quadrant pain, and bloody diarrhea. Respiratory symptoms have been reported in up to 70 percent of persons infected with *S. mansoni* but less frequently in those infected with *S. haematobium* (Bethlem, *et al*., 1997; Cooke *et al*., 1999). Other observed symptoms include tender hepatomegally and splenomegally in one third of the cases. Though most of the patients have eosinophilia and positive serologic tests not all patients shed eggs.

In humans, acute disease is associated with a massive dissemination of granulomas around the eggs, especially in the liver, lung, pancreas and lymph nodes. Acute, diffuse, severe hepatitis and enterocolitis have also been described. The humoral response (IgG, IgM and IgE) to egg and worm antigens has been shown to be equivalent in patients with acute and chronic schistosomiasis (Kanamura *et al*., 1979; Rabello *et al*., 1995). High
levels of IgG and IgM antibodies to Keyhole Limpet Hemocyanin (KLH) have also been
detected in acute patients and this has thus become one of the simple diagnostic tools
with high sensitivity and specificity, for differentiation between acute and chronic
schistosomiasis (Alves-Brito et al., 1992; Rabello et al., 1995).

Cellular responses of acute patients clearly differ from those observed in most chronically
infected patients (Gazzinelli et al., 1985). Acute patients express considerably higher in
vitro responsiveness than do intestinal chronic patients, especially in regard to responses
of their peripheral blood mononuclear cells (PBMC) to soluble schistosoma egg antigens
(SEA). Katayama fever due to *S. mansoni* or *S. haematobium* is rarely seen in chronically
exposed populations, possibly owing to under diagnosis or due to in-utero sensitization
(Hatz, 2005). Acute schistosomiasis is however common, in tourists, travelers, and other
people accidentally exposed to transmission (Jelinek et al., 1996). Katayama fever due to
*S. japonicum* does however occur in people living in endemic areas and with a history of
previous infections. Its manifestations can be severe with persistent fever,
organomegally, and cachexia, which can evolve rapidly to hepatosplenic fibrosis and
portal hypertension (Chen, 1993).

Treatment has mainly been with the use of Praziquantel since it works exclusively against
the adult worms although Oxamniquine has been used in some instances (Sleigh, 1991;
Chen and Mott, 1989). Most patients recover spontaneously after 2–10 weeks, but some
develop persistent and more serious disease with weight loss, dyspnoea, diarrhoea,
diffuse abdominal pain, toxaemia, hepatosplenomegaly and widespread rash.
1.2.3 Chronic Schistosomiasis

Chronic schistosomal disease mainly affects individuals with longstanding infections in poor rural areas. Immunopathological reactions against schistosome eggs trapped in the tissues lead to inflammatory and obstructive disease in the urinary system (S. haematobium) or intestinal disease, hepatosplenic inflammation, and liver fibrosis (S. mansoni, S. japonicum) (Gryseels et al., 2006). In urinary schistosomiasis, the eggs of S. haematobium provoke granulomatous inflammation, ulceration, and pseudopolyposis of the vesical and ureteral walls (Cheever et al., 1978). Common early signs include dysuria, pollakisuria, proteinuria, and especially haematuria (Gryseels, 1989). Unfortunately this is sometime confused with menstruation in girls and even a coming of age in boys (Jordan, 2000). Some of these chronic lesions can develop to fibrosis or calcification of the bladder and lower ureters, resulting in hydroureter and hydronephrosis. Chronic compression can eventually lead to parenchymal damage and kidney failure (Gryseels et al., 2006). Borojevic (1992) regards the chronic phase of murine schistosomiasis as predominantly Th-1 mediated. Studies in murine schistosomiasis also demonstrate that the development of fibrosis requires the production of the profibrotic cytokines IL-2 and IL-4, and is suppressed by IL-12 and IFN-γ. (Cheever et al., 1998; Correa-Oliveira et al., 1998).

Intestinal schistosomiasis is caused by Schistosome eggs migrating through the intestinal wall where they provoke mucosal granulomatous inflammation, pseudopolyposis, microulcerations, and superficial bleeding (Cheever et al., 1978). It is characterized by chronic or intermittent pain and discomfort, loss of appetite and diarrhea with or
sometime without blood (Gryseels, 1989). Inflammatory hepatic schistosomiasis is an early reaction to ova trapped in the presinusoidal periportal spaces of the liver. Granuloma formation is an inflammatory response against the parasite eggs that are trapped in the host tissues. The intensity of this response can either be modulated by the host (Goes et al., 1991) or by the parasite factors (Boulanger et al., 1992). There are several adult worm antigens that do cross react with the egg components and are thus able to modulate the immune responses against eggs (Dunne et al., 1988). Inflammatory hepatic schistosomiasis can either be caused by *S. mansoni*, *S. japonicum*, and *S. mekongi* (Gryseels et al., 2006). Bleeding from gastro-oesophageal varices is the most serious, commonly fatal, complication of fibrotic hepatic schistosomiasis. In *S. mansoni* infections, it tends to recur and grow more severe over time; in *S. japonicum*, bleeding is sudden and massive in many cases (Chen, 1993). Repeated or occult bleeding can lead to anaemia, hypoalbuminaemia, cachexia, and growth retardation (Gryseels et al., 2006).

1.3 Diagnosis

Detection of schistosomes eggs in the feces or urine is usually considered diagnostic of schistosomiasis and so far it remains the gold standard (Feldmeier and Poggensee, 1993). Quantitative egg counts after standardised urine filtration or in calibrated faecal thick smears are especially useful for epidemiological surveys and control since they correlate well with worm burdens and morbidity (Jordan and Webbe, 1993). Formalin-based techniques for sedimentation and concentration have been used to increase the diagnostic yield and incase of *S. mansoni* or *S. japonicum* eggs may be observed in stool specimens of 2 to 10 mg with or without suspension in saline (Garcia et al., 1999). However the
individual egg counts should not be over interpreted as a measure of disease since they have been shown to vary substantially within and between stool and urine samples (De Vlas et al., 1992). To rule out *S. japonicum*, miracidium-hatching test has also been extensively used by the public health workers in China (Cheever, 1978). For those patients who do show typical clinical presentation but have negative urine and feces specimens, a biopsy of bladder or rectal mucosa is usually done for diagnosis.

Antibody detection may also be carried out in some circumstances though it is limited due to the persistence of antibodies after parasitological cure. In such circumstances as Katayama fever where there are no eggs, a positive serologic test may be diagnostic (Tsang and Wilkins, 1997). Serologic testing is also applied in regions of low endemicity where the individual patients have low egg burdens (Al-Sherbiny et al., 1999). Though immunodiagnosis is still considered vital in some instances for diagnosis of schistosomiasis, the commercially available immunodiagnostic kits are not as sensitive as multiple fecal examinations and are also less specific (Tsang and Wilkins, 1997). Though quite sensitive, antibody-based assays cannot distinguish history of exposure from active infection and they have also been shown to cross-react with other helminths and are thus not easily applicable under field conditions (Feldmeier and Poggensee, 1993; Rabello, 1997). Immunodiagnosis in schistosomiasis is however important for diagnosis in travellers, migrants, and other occasionally exposed people as well as in incidence studies in children and in low-transmission or post-control settings (Tsang and Wilkins, 1997). Most of these immunodiagnostic routine techniques detect IgG, IgM, or IgE against soluble worm antigen or crude egg antigen by ELISA, indirect haemagglutination, or
immunofluorescence. It has been noted that most of these assays have positive results for at least 2 years after cure and in many cases much longer (Rabello et al., 1997).

Detection of circulating adult worm and egg antigens in serum or urine of infected individuals may also be applied through the use of labeled monoclonal antibodies (Van Lieshout et al., 1997). This is however not appropriate in clinical applications as antigen detection in serum is not very sensitive with light infections. It is however a valuable research tool for epidemiological and therapeutic studies since it is a specific, direct and a stable measure of worm burdens (Polman, 2000). Even the less specific urine-based antigen detection assays have been shown to have the potential for the development of field-applicable reagent strips (van Dam et al., 2004). It is estimated that an immunoblot assay for the detection of adult-worm antigens have up to 95 percent sensitivity and 100 percent specificity (Wang et al., 1999).

Evidence of peripheral-blood eosinophilia, anemia (iron-deficiency anemia, anemia of chronic disease, or macrocytic anemia), hypoalbuminemia, elevated urea and creatinine levels, and hypergammaglobulinemia may also be used as an additional supportive laboratory evidence of schistosomiasis. In a hospital setup, cystoscopy and endoscopy are used to visualise bladder lesions and oesophageal varices (AbdelWahab et al., 1993). Laparoscopy and wedge biopsy can also be used to reveal the macroscopic and histological appearance of granulomatous inflammation or periportal fibrosis (Hayashi et al., 2000).
1.4 Control of Schistosomiasis

Control of schistosomiasis is a realistic option as endemic countries such as Brazil, China, the Philippines and Egypt, have been able to sustain national control programmes for a prolonged period and have succeeded in reducing morbidity to very low levels. Countries such as Caribbean Islands, the Islamic Republic of Iran, Mauritius, Morocco, Puerto Rico, Tunisia and Venezuela, are nearing elimination or have already achieved this goal (WHO, 2002) although there is almost non existent control measures in sub-Saharan Africa. Brazil, Egypt and Sudan have had less schistosomiasis related morbidity compared to sub-Saharan Africa where it has been suggested that deaths could be as high as 200,000 per year due to schistosomiasis associated ills (Gryseels, 1989). Different countries initiated control programs before or during the 1980s and some progress has been made. Unlike sub-Saharan Africa with a great number of people infected or at the risk of infection, formerly endemic countries such as Asia and America, have reduced the number of infected people and the risk of infection due to successful control programmes (Engels et al., 1993).

Since the introduction of modern schistosomicides and particularly praziquantel, strategies employed in schistosomiasis control have been changing over the past few decades (Gryseels et al., 2006). WHO has recommended the control of early and late morbidity associated with schistosomiasis infection as the first objective (WHO, 2002). Improved access to sanitation and clean water, appropriate health education, and treatment, snail control measures as well as vaccine development are required for permanent solutions. As it has been shown in Japan, Schistosomiasis can in principle be
eliminated by behavioural changes, sanitation, and safe water supply (Minai et al., 2003). Behavior change has been observed to be difficult without other options of getting water but educational programmes can improve knowledge about the disease and health-care seeking (Engels et al., 1993; Sow et al., 2003).

### 1.4.1 Elimination of vector snails

The control of snails with molluscicides and toxic chemicals is expensive as well as complex. For its sufficient application, substantial human and material resources are needed as well as detailed epidemiological and malacological surveillance. Snail populations can be greatly reduced but rarely eliminated, so regular and long-term retreatment is necessary. In the past, the toxicity of molluscicides for other aquatic organisms, including fish has given rise to ecological and economic concerns. Large-scale chemical snail control is still used in Egypt and China, but owing to the success of population-based chemotherapy, its cost-effectiveness has been questioned (Zhang and Wong, 2003). Snail control can also be pursued by physical measures or biological competitors, but such methods are not easy to put into practice (Cowie, 2001; Laamrani and Boele, 2002).

Today, the identification of early snail infection is considered the most relevant outcome for linking snail infection to water contamination (King et al., 2006). This is so because the mortality of infected snails can be higher after the cercarial shedding and thus measuring prepatent infection (incubation before shedding) has been considered more suitable than measuring patent infection for quantitative assessment of the impact of control programs on human-to-snail transmission (Woolhouse, 1989). Detection of snail
infection throughout prepatency has been done through PCR amplification of tandem repeated DNA sequences (Sm1–7 repeat of *Schistosoma mansoni* and DraI repeat of *S. haematobium*). Large-scale monitoring of field snails by DraI-PCR has been applied in Msambweni area of Kwale District, Coast Province, Kenya to detect prepatent *S. haematobium* infections in bulinid snails (Hamburger, 2004). In these kind of studies, 30–50% of field snails recovered were found to be infected with *S. haematobium* even when community-wide therapy was applied suggesting a high residue of water contamination by parasite eggs despite the fact that there were significant reductions in mean community infectious burden following treatment (Hamburger *et al.*, 2004). Thus, monitoring of continuing transmission potential (measured as snail prepatent infection rates) could help to select the most effective modes of snail control and chemotherapy timing. This could also enable rapid, performance-based decisions about applying additional snail control measures such as focal molluscicide application for prevention of recurring infection and disease (King *et al.*, 2006).

Snails can also be controlled biologically using competitor snails. Competitor snails are voraciously feeding snails, species with higher fecundity rates, snails resistant to parasites, have a longer lifespan and are harmless to other animals and the surrounding crops (Giovanelli *et al.*, 2003). *Biomphalaria glabrata* and *B. straminea* has been controlled by introducing *Thiara granifera* and *Melanoides tuberculata* (Pointier, 1993). The snail *Physa acuta* has also been used in biological control especially for *Bulinus truncates* (Bakry and Abd-el-Mone, 2005). The use of molluscivorous fish (predators of schistosome-transmitting snails) such as *Sargochromis codringtoni* and *Geophagus*
*brasilensis* has also been appreciated (Weinzettl and Jurberg, 1990; Slootweg *et al.*, 1994; Makoni *et al.*, 2005;). Other fish that can be considered to be molluscivorous include; North American crayfish *Procambarus clarkii* known to prey on *B. pfeifferi* and *B. glabrata*, and the African catfish, *Clarias gariepinus*, which is considered to be both molluscivorous and carnivorous (Makoni *et al.*, 2005).

### 1.4.2 Chemotherapy

In the absence of a vaccine, water sanitation and efficient vector control mechanisms, treatment and control of schistosomiasis relies mostly on the single drug praziquantel (PZQ;2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino(2,1-alpha) isoquinolin-4-one) (Doenhoff and Pica-Mattoccia, 2006). Though more expensive than praziquantel, oxamniquine (6-hydroxymethyl-2-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline), is also available although it’s bioactivity is restricted to *S. mansoni*, and has largely been replaced in favor of the more cost-effective PZQ (Beck *et al.*, 2001). A single oral dose of 40-60 mg/kg has been shown to achieve cure rates of up to 60-90% (Doenhoff and Pica-Mattoccia, 2006) and it has been established as a safe and effective drug (Cioli and Pica-Mattoccia 2003). At either the community or individual level, PZQ is the recommended drug for the disease treatment, (WHO, 2002).

The exposure of the worms to PZQ causes a massive influx of calcium (Pax *et al.*, 1978) contraction of the masulature (Fetterer *et al.*, 1980) and disruption of the tegument (Becker *et al.*, 1980). Though the precise molecular targets are not clear, there is accumulated evidence on the involvement of calcium channels which is supported by recent data with calcium-channel blockers and the actin depolymerization agent,
cytochalasin D (Pica-Mattoccia et al., 2007). PZQ has been found to have a decreasing efficacy against immature parasites relative to adult worms (Gonnert and Andrews 1977; Sabah et al., 1986). Thus a second treatment of PZQ, four to six weeks after the initial dose, is recommended in cases of clinical use to remove any parasites that have matured in the intervening period (Renganathan and Cioli, 1998). The greater use of PZQ through control programs such as the Schistosomiasis Control Initiative may quicken resistance to PZQ (Fenwick, 2006; Fenwick and Webster, 2006). PZQ has already become less effective with other fluke diseases (e.g. clonorchiasis in South East Asia) (Tinga et al., 1999; Keiser and Utzinger, 2004). The search for alternative chemotherapies for use either alone or, perhaps, in combination with PZQ are thus further encouraged.

1.4.3 Vaccination

The struggle for a schistosomiasis vaccine is considered a realistic aim since previous studies have shown that human populations in endemic areas invariably develop some degree of protection naturally (Butterworth et al., 1985; Hagan et al., 1991). Considerable effort has been devoted to the development of vaccines against schistosomiasis and some of the several antigens judged to be potential vaccine candidates have been tested in animals with varying results (Bergquist and Colley, 1998; Hewitson et al., 2005). The recombinant rShGST-28 (Bilhvx; Eurogentec, Herstal, Belgium) has already undergone phase I and II clinical trials though questions still remain about the feasibility, applicability, and relevance of schistosomiasis vaccines (Gryseels 2000; Capron et al., 2005). Vaccination with radiation-attenuated (RA) cercarie is one of the most reproducible protocols (Coulson, 1997) resulting in 50 to 80% reduction in challenge worm burden. Due to safety reasons, this vaccine has not
been tested in human volunteers (Coulson, 1997) but it could form the basis for a recombinant vaccine if the relevant immune mechanisms and the protective antigens are known (Eberl et al., 1999). There is a great challenge in trying to mimic as well as accelerate the mechanisms that are observed in humans living in endemic areas in experimental animals (Bergquist et al., 2002).

Some of the antigens that have been isolated and tested as vaccine candidates include two different multiple antigenic peptides (MAP), Sm23, an integrated membrane antigen, and triose phosphate isomerase (TPI) (Harn et al., 1995). Other antigens are full-length proteins: paramyosin (Pearce et al., 1988; Flanigan et al., 1989; Sher et al., 1991), IrV5 (Soisson et al., 1993), GST (Capron et al., 2001) and Sm14, a fatty acid-binding protein (Brito et al., 2000). Glyceraldehyde phosphate dehydrogenase (GAPDH) that was first reported by Goudot-Crozel et al., (1989) and then later on by Argiro et al., (2000) has also been researched on.

The golden standard for anti-schistosome vaccine development is the reduction in worm numbers. However since schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability appears also entirely relevant as is S. haematobium GST vaccine, Bilhvx (Capron et al., 2005). Capron et al., (2001) has suggested that vaccination can be either targeted towards the prevention of infection or to the reduction of parasite fecundity. Previous immunization experiments of mice with one dose of irradiated cercariae results in 50–70% protection which can be increased to over 80% with two or three immunizations (Smythies et al., 1996). IL-12 has been shown to be a useful adjuvant for use in vaccination when used
together with an antibody that blocks the IL-10R. In these kind of experiments, sterile immunity against infection has almost been attained in mice that have either the gene for the immuno-regulatory cytokine IL-10 disrupted or in which IL-12 is used as a defined adjuvant by vaccination with radiation-attenuated cercariae (Wynn, 1996).

Unfortunately, the individual recombinant antigen vaccines tested in animal models have failed to achieve the protection levels predicted to be necessary for an efficacious and affordable human vaccine (Todd and Colley, 2002). DNA plasmids encoding single or multiple genes have several attractive attributes and have also been explored as vaccines for schistosomiasis (Da’dara et al., 2001; Shalaby et al., 2003). Berghist et al., 2002 noted that immunization of experimental animals with defined schistosome genes or recombinant antigens is consistently less effective in conferring protection than vaccination with attenuated cercariae regardless of the specific antigen under study or the laboratory performing the study. It has been demonstrated that the decreased efficacy of DNA vaccines, and probably recombinant proteins compared to irradiated cercariae vaccinated animals could largely be due to differences in the inflammatory responses they are capable of provoking (Shalaby et al., 2003).

**1.4.3.1 Radiation attenuated (RA) vaccine**

This is the most studied model of schistosome immunity induced by live attenuated parasites (Coulson, 1997). Immunity induced by the RA vaccine has been shown to have a strong T-cell component (James, 1999) and there is suggestive evidence that killing of the larval schistosomulae is mediated by the macrophages and the endothelial cells through the action of interferon λ (IFN-λ) (Coulson, 1997). However, it is not clear if the
killing in the lungs occurs as a result of nitric oxide (NO) release or whether migration is blocked by a focus of non-cytotoxic inflammatory cells. In animal models that have received multiple immunizations with attenuated parasites, antibodies have been shown to play an important role in protective immunity. This has been demonstrated through passive transfer experiments in which significant levels of protection against challenge infection has been achieved through passive immunization (Mangold and Dean, 1986).

In C57BL/6 mice vaccinated with RA vaccine, 60-70% protection is routinely achieved with a single exposure and the mechanism of immunity is thought to be cell mediated. The CD4+ T cells of the Th1 subset play a central role in the pulmonary effector responses, which are orchestrated by the cytokine interferon gamma. Both IgG and IgM antibody isotypes specific to parasite antigens; RAP (material released during cercarial transformation into schistosomula from hours 0 to 3), SLAP (soluble proteins from lung schistosomula), and SEA (Soluble egg antigen), are detected after RA vaccination (Kariuki et al., 2004). Their levels have been shown to be dependent on the amount of attenuating radiation as cercariae exposed to 20 kilo radiation (krad) have been shown to be better inducers of protection in 3 out of 4 experiments as compared to those exposed to 60 krad (Yole et al., 1996). Simultaneous induction of both humoral and cell-mediated immunity is needed for effective vaccination against schistosome (Jankovic et al., 1999).

In the RA vaccine trial by Kariuki et al., (2004), antibody titers observed indicated an initial rise to a peak of IgM reactive with larval secretions RAP after two vaccinations before declining. IgM levels rose rapidly at 6 weeks post challenge with an observed
progressive rise of IgG levels. IgG levels in response to RAP at challenge gave the best correlation coefficient (0.43) between antibody response and protective immunity suggesting that this isotype mediates the effector response. Levels of IgG rapidly declined after vaccination with no boost provided by the challenge before egg deposition began.

1.5 Immune responses to schistosomiasis

There have been several observations that people living in endemic areas acquire some form of immune resistance after years of exposure (Butterworth, 1993). There is also the evidence in human population of both natural resistance (Viana et al., 1995) as well as acquired immunity against schistosome (Hagan et al., 1991; Dunne et al., 1992). Since the parasite does not replicate in the definitive host, a partial reduction in the worm burden would be beneficial in reducing transmission and morbidity (Chan et al., 1997).

The identification of the relevant protective immune mechanisms in humans is a challenge due to the uncertainties of the worm burden acquired and also unlike experimental animals, they are continuously exposed to schistosome cercariae (Butterworth et al., 1994). Humans are also highly susceptible to other parasite infection and due to their variation in the nutritional status and behavior, the immune mechanisms involved are further complicated (Marti et al., 1987; Gryseels, 2000). Some of the recent studies with infected humans have indicated that the response to schistosomes could be a Th2-cell response, high IL-10 and IgG4 antibody with a low IgE reactions (Maizels and Yazdanbakhsh, 2003).
Cellular immune responses induce most of the schistosomiasis related pathology. The granulomatous reactions usually observed around the eggs are orchestrated by CD4-positive T cells and involve eosinophils, monocytes, and lymphocytes (Cheever et al., 2000). In a mice model, T-helper-1 reaction in the early stages of infection shifts to an egg-induced T-helper-2-biased profile, and the resulting imbalances between these responses lead to severe lesions (Pearce, 2005). These similar mechanisms are thus thought to be the reason behind fibrotic pathology in human beings (Abath et al., 2006).

Previous studies have demonstrated the role of the host's humoral response in the elimination mechanisms of *S. mansoni* from mice submitted to different immunization protocols (Mangold and Dean, 1986; Mangold and Dean, 1992). In various studies, it has been shown that there is a balance between the level of effective anti-schistosomula antibodies and the presence of blocking antibodies (Hagan et al., 1991). The latter (blocking antibodies) are sometimes most easily demonstrated as antibodies to egg antigens that cross-react with epitopes present in the schistosomula tegument (Butterworth et al., 1988). High levels of IgE against adult worm or larval antigens has been associated with resistance to reinfection while high levels of IgG4 and IgM antibodies against egg antigens generally parallel susceptibility (Hagan et al., 1991; Rihet et al., 1991). A correlation between resistance and elevated levels of IgA against a schistosome vaccine candidate (Sm 28 glutathione-S-transferase) has been reported (Grzych, et al., 1993) and further studies with other antigens have demonstrated that higher levels of IgE against a 22-kD schistosomula moiety (Dunne et al., 1992) and
higher levels of IgM against a 68-kD adult schistosome antigen also correlate with resistance to infection.

In mice, acute phase response has been shown to be mediated by the release of certain cytokines. In murine intestinal schistosomiasis, there is a type I (Th-1 type) immune response where increased interferon-gamma (IFN-γ) predominates in early infection, but this type of immune response changes as egg production and tissue reaction begins (Correa-Oliveira et al., 1998). As infection progresses, there is a decrease in IFN-γ which is accompanied by an increase in IL-10 and granuloma formations. The earliest hepatic granulomas, therefore, form in a Th-1 environment, with down-regulation of Th-1 and up-regulation of Th-2 responses six weeks after infection (Todt et al., 2000). The granuloma is conceptualized as a Th-2 dominant reaction, but under some conditions, Th-1 granulomatous response may be predominant and damaging (Rutitzkky et al., 2001). However, in chronic infections which are considered as 20 weeks and over, these responses are less marked (Henderson et al., 1992).

### 1.6 Protective Immunity

The acquisition of effective immunity against schistosomes has so far been difficult to prove, since the decrease in infection rates observed after adolescence can also be explained by reduced water contact. Comparative studies of re-infection after curative treatment have shown that children are far more susceptible than adults and that these differences cannot be explained by differing water-contact patterns (Gryseels, 1996; Woolhouse and Hagan, 1999). Other observations in people and animals suggest that
acquired immunity is mediated by IgE against antigens of larvae and adult worms, which trigger eosinophils to release cytotoxins targeting schistosomulae (Butterworth, 1993). Thus the observed slow development of acquired immunity is thought to be due to blockage of the IgE receptors by excess anti-schistosome IgG₄ and possibly other immunoglobulin isotypes in the first years of infection.

Humans have shown acquired immunity to schistosome infection and individuals are usually classified as either being susceptible or resistant to natural infection by schistosomes (Butterworth et al., 1994). Though the host responds to S. mansoni infection with an immune response that does limit subsequent challenge infection, there is little or no effect on established adult worms. This is so because the schistosome parasite is most susceptible to immune elimination during the skin and lung stages of development and least susceptible as an adult parasite (James, 1992). However, through haptenation (McCormick and Damian, 1987) or through treatment with praziquantel, adult schistosomes can be made vulnerable to immune elimination both in vitro and in vivo. Studies done on cellular and humoral immune responses to Schistosoma mansoni antigen preparations in individuals presumed to be susceptible or resistant to reinfection after chemotherapeutic cure have shown that resistance to reinfection is an age-related phenomenon, with most people in endemic areas becoming resistant, or expressing their resistance during their second decade of life (Caldas et al., 2000). This resistance is usually attributed to immunity rather than physiologic or behavioral changes with age as it appears to be unrelated to the degree of contact with cercariae containing water (Barbour, 1985).
Killing of the schistosome parasite after primary infection that is associated with IgE does mostly occur after the schistosomula has passed through the lungs since induction of a primary IgE response normally requires 5–10 days. This however still remains suggestive as the actual target and site for parasite attrition are speculative (King et al., 1997). In a rat model which is a non-permissive host, the killing of schistosomulae of challenge infection is by antibody-dependent cell-mediated cytotoxic (ADCC) response (Capron and Capron, 1986). In baboons and unlike mice, adult worm-specific IgE is uniquely associated with acquired immunity to S. mansoni infection (Nyindo et al., 1999).

1.7 Animal models for human Schistosomiasis

There are several animal models that have been used to study the basic biology, immunology and pathogenesis of schistosomiasis (Sturrock, 1986). Susceptibility to primary infection with Schistosoma mansoni has been shown to vary among different species and strains of laboratory animals (Warren and Peters, 1967). Because of their ease of availability, fast breeding and development of disease on experimental infection, mice have been used in most experimental Schistosoma mansoni infection (Nyindo and Farah, 1999). The disadvantage with using mice is that extrapolation of data from mice to humans is associated with some difficult due to the anatomical, genetic and immunological differences that exist between the two species. In addition, murine studies have been performed in highly inbred strains of mice, and might not reflect the heterogeneity of response expected from human populations. However, since the disease
progress in mice is to some extent similar to that seen in humans, murine schistosomiasis remains the most studied experimental model (Cheever et al., 2002).

Baboons represent suitable models for the study of human schistosomiasis infection as evaluated by Nyindo and Farah (1999). This is for several reasons: (1) baboons are anatomically, genetically and immunologically more similar to humans than are rodents, (2) they acquire natural infections and are highly susceptible to experimental infections, (3) they develop hepatic and intestinal pathology, modulate this pathology and acquire protective immunity, as do humans, (4) baboons infected in the wild show an age-dependent prevalence, with high rates of infection in juveniles and young adults, (5) they adapt readily to changes in their environment, can give birth twice every 18 months, can live for 20 years and can attain up to 20 kilograms body weight. Their moderate size and the capacity to monitor disease at different time points in an individual animal by surgical manipulations, as well as ease of perfusion for adult worm recovery makes the baboon a good model.

Baboons like mice and humans develop resistance to infection with S. mansoni and this protection has been thought to be immunologically mediated and related to increased production in the serum of adult worm-specific IgG (Suzuki and Damian, 1981). Like mice, baboons can also be immunized with S. mansoni irradiated cercariae and the repeated exposure of baboons to irradiated cercariae stimulates from 30% to 90% protection to challenge infection with S. mansoni (Yole et al., 1996). There is also an association of parasite-specific IgE and protection among primates infected with
schistosomiasis, along with pathology, anatomy, and genetic make-up thus indicating that baboons provide an excellent permissive experimental model for better understanding the mechanisms of innate and acquired immunity to schistosomiasis in humans (Nyindo et al., 1999).

Some of the drawbacks associated with the use of baboon as a model for schistosomiasis infection includes; (1) baboons are expensive to buy and maintain in captivity; (2) it is necessary to obtain the services of qualified primate medicine veterinarians to look after the health of the animals and to perform procedures for specific experiments; (3) there is an urgent need to develop baboon or non-human primate specific reagents because some of the human reagents might not work in baboon assays (Nyindo and Farah, 1999). Despite these drawbacks, the baboon will probably continue to be the non-human primate model of *S. mansoni* infection. Chimpanzees which are much closer genetically and physiologically to humans do become infected in the wild with schistosome (Renquist et al., 1975; Abe et al., 1993) developing pathology that is indistinguishable from that in human patients and have thus also been used in some experiments (Sadun et al., 1966; von Lichtenberg et al., 1971).

### 1.8 Passive immunization

Mangold and Dean (1992) have previously shown that partial immunity against *Schistosoma manssoni* infection can be passively transferred to C57Bl/6 mice with serum from mice that have been multiply immunized with cercariae irradiated with 50 kilorads of gamma radiation. It has been demonstrated that serum from irradiated cercarie
immunized rabbits partially protects mice against a *S. mansoni* challenge (Bickle *et al.*, 1985). Different animals have been used as the source of this serum for passive immunisation experiments. Use of large animals such as rabbits and baboons has the advantage of providing large amount of serum from a relatively small number of animals that can be repeatedly boosted. These previous experiments have shown that 1) the Fc portion of the antibody molecule is necessary for passive protection, 2) the timing of injection is crucial and 3) IgG antibodies are specifically involved in protection (Mangold and Dean, 1992).

Role of humoral immunity to *S. mansoni* infection has been examined by employing passive transfer system (Ford *et al.*, 1984; Mangold and Dean, 1986; Jwo and Loverde, 1989) with either fractionated or non fractionated serum. Antibody specificity rather than quantity could be more relevant to protective immunity as no consistent association between antibody titer and level of resistance is apparent (Richter *et al.*, 1995). The IgG isotype particularly IgG1 seems to be protective and may be synergistically enhanced by the presence of IgM (Jwo and Loverde, 1989). The parasite stage that is the target for antibody mediated attack and the site of immune elimination are controversial (Coulson and Mountford, 1989; Richter *et al.*, 1995) although Sher *et al.*, (1984) demonstrated that the newly transformed schistosomula could be the target of antibody mediated attack. Serum administered one day pre-challenge, 4th to 7th day or day 15th post-challenge have been shown to be less effective, most effective and totally ineffective respectively (Mangold and Dean, 1992). Both IgM & IgG isotypes of sera from humans with chronic
Schistosoma mansoni infections has been shown not to protect when administered greater than or equal to 24 days after challenge (Jwo and Loverde, 1989).

1.9 Statement of the problem

An effective vaccine will be able to sustain the protective immunity for a significant period of time. The vaccine would be required to induce a strong humoral response that can be sustained for long. Previous studies have shown that vaccination with Radiation Attenuated vaccine induces a strong humoral response but the antibody titer soon drops a few weeks after the last vaccination with a corresponding drop in the level of protection after challenge infection (Kariuki et al., 2004). This could imply that there is an inability to sustain antibody titer for long. It could also imply that the role of antibodies in mediating protection is not yet understood.

1.10 Research question

Can hyper-immune serum from baboons confer protection in mice against pre-liver-lung stage schistosomulae and or the migrating schistosome larvae?

1.11 Null hypotheses

Ho₁: Hyper-immune serum from baboons does not protect mice against pre-liver-lung stage schistosomulae.

Ho₂: Hyper-immune serum from baboons does not protect mice against the migrating schistosome larvae.
1. 12 Objectives

1.12.1 Main objective

To determine the role of humoral immunity in mice infected with *Schistosoma mansoni*.

1.12.2 Specific objectives

i. To determine the effect of antibodies generated from RA vaccinated baboons on the number and nature of schistosome worms.

ii. To determine the effect of antibodies generated from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver.

iii. To determine the effect of antibodies generated from RA vaccinated baboons on the size and number of granulomas in the liver.

iv. To compare the levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) in mice.

1.13 Justification and expected outcome

Radiation attenuated (RA) vaccine evaluated in the olive baboon, *Papio anubis* (Kariuki *et al.*, 2004), have shown 86% protection against challenge with 1,000 normal cercariae after vaccination, five times (5×), with 9,000 RA cercariae at 4 week intervals. Given the efficacy of RA vaccine in rodents and the protection levels it elicits in baboons, it can serve as an important paradigm for human vaccine against schistosomiasis (Coulson, 1997). In this vaccine trial in baboons, the difference in mean worm burdens between the 3-week challenge controls and the VC3 (vaccinated and challenged 3 weeks post last vaccination) group at perfusion amounted to 72% protection, while that between the 12-
week challenge controls and the VC12 (Vaccinated and challenged 12 weeks post last vaccination) was 53% (Kariuki et al., 2004). If the protection due to this vaccine is antibody mediated, the reason protection is not sustained could be the inability to maintain antibody titers.

There is thus need to evaluate the role of the antibodies in mediating protection in order to formulate recombinant antigen vaccines that will maintain high antibody titers for long. Since antibodies could be involved in protection against cercariae challenge, a reduction in schistosome liver and faecal eggs, adult worms, size and number of granulomas after passive immunization, will demonstrate the active role of antibodies against cercariae challenge. Differences in level of protection due to different time points of serum injection, will give a better understanding on the schistosome life-cycle stage (migrating larvae or pre-liver lung stage schistosomulae) most susceptible to antibodies. This information is important when developing more effective vaccines that can maintain high antibody titers for long.
CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental Design

There were two experiments each with 15 adult C57BL/6 mice aged 6-8 weeks at the start of the experiment. In each experiment, mice were challenged with 200 *S. mansoni* cercariae and then divided into three groups each of 5 mice. Group one received 500 µl of hyper-immune serum, group two 500 µl of infection serum and group three was not immunized. In experiment one, mice were immunized on day 7 and boosted on day 14 after challenge while in experiment two, mice were immunized on day 3 after challenge with no boost. The research protocols were reviewed and approved by the Ethical Review Board of Institute of Primate Research (IPR) (Nairobi, Kenya).

2.2 Intermediate host snails and parasites

*Biomphalaria pfeifferi* snails obtained from a schistosome free dam in Kibwezi district, Kenya were used in these experiments as the intermediate host for *Schistosoma mansoni*. They were screened for any pre-existing schistosome infection by exposing them to strong light (100 watts lamp) for two hours for six consecutive weeks (Frandsen and Christensen, 1984). Those snails that were negative (not shedding cercariae) were maintained in snail tanks within the snail colony at Institute of Primate Research (IPR) and water was changed twice a week. They were fed on soft lettuce and the temperature maintained at 25 °C -27 °C. The *S. mansoni* parasites used as a source of schistosome eggs in this experiment were maintained in chronically infected baboons, PAN (*Papio*
anubis) 1875 and PAN 1873, at IPR primate colony. They were originally from schistosome eggs collected in stools from children in Kibwezi district Kenya.

2.3 **Definitive host C57BL/6 mice**

Adult male and female C57BL/6 mice (n =30) aged (6-8) weeks at the start of the experiment and bred at International Livestock Research Institute, (ILRI, Nairobi Kenya) Animal House Breeding stock were used. The animals which were acclimatized for two weeks before experimental infection were housed in groups of five per a standard plastic cage with stainless steel cover lids and wood shavings as beddings. They were fed daily on commercial pellets (Rodents pellets®, Unga Feeds Ltd, Kenya) and water *ad libitum* within the rodents’ animal house facility at IPR (Institute of Primate Research).

2.4 **Source of serum for passive immunization**

Serum tested in this experiment was from a previous baboon vaccination experiment performed at the Institute of Primate Research Karen Nairobi Kenya by Kariuki *et al.*, (2004). Juvenile Olive baboons (*Papio anubis*) were vaccinated five times with 9,000 RA cercariae at 4- week intervals and challenged 3 weeks after the last vaccination with 1,000 cercariae. Samples of 15 ml of blood obtained from vaccinated animals before challenge infection were incubated at 37 °C for 1-2 hours then stored at 4 °C overnight to allow complete coagulation. Serum was recovered by spinning the blood samples on a bench top centrifuge 1500 rpm for 15 minutes, and serum aliquots stored at -20 °C.
2.5 Passive immunization

Mice were immunized through an intravenous injection of the serum through the tail vein (Mangold, and Dean, 1986; Mangold and Dean, 1992). Group one was immunized with hyper-immune serum; five times vaccinated baboon serum (5× VBS) 0.5ml/mice and group two with 0.5 ml/mice of infection baboon serum (IBS) from a previous baboon vaccination experiment as explained in 2.4 above. Mice in the control group were not immunized. Immunization was done on the 7th and 14th day after challenge in the first experiment, and on the 3rd day in the second experiment using. Mice to be immunized were isolated in a small cage and warmed with a 100 watt lamp to increase blood supply to the tail. Each mouse was then moved into the restraining devise and a portion of the tail (about 1.5 cm below the base) swabbed with alcohol. A 1.0 ml syringe fitted with a 26 gauge needle and containing either the immune serum or infection serum was guided into one of the tail veins and the serum slowly delivered. The needle was then removed after a few seconds and the immunized mouse returned to its cage.

2.6 Parasitological techniques

2.6.1 Infection of snails with miracidia

*Biomphalaria pfeifferi* snails were infected with miracidia hatched from *Schistosoma mansoni* eggs that were recovered from faecal material of chronically infected baboons. The faecal matter was homogenized with saline solution and the slurry sieved through 600µm and 125 µm test sieve (Arther Thomas CO. USA) into urine glass jars. The slurry was then left to sediment for 30 minutes in the dark after which the supernatant was
poured out and the pellet resuspended in saline three times until the supernatant became clear. After the last wash, water was added and the supernatant poured. Sediments were then transferred into a section of a petridish and the remaining section gently filled with water. This petridish was then exposed to bright light (100 watts lamp) for one hour to facilitate the hatching of miracidia. Using a dissecting microscope, 3-5 miracidia were picked from the petridish using a pipette mounted onto a rubber bulb and dispensed into beakers containing approximately 1ml of water. A single snail was then placed into each beaker and left for 30 minutes to facilitate miracidia penetration. The snails were then transferred into a single snail tank for 5 weeks for cercariae production to take place and a week before scheduled cercaric shedding, the infected snails were kept in the dark (Stirewalt and Uy, 1969).

2.6.2 Infection of mice

After 5 weeks, cercariae were shed from infected snails (described in 2.5.1) by exposing the snails to bright light (100 watts lamp) for two hours. Using a dissecting microscope, 200 cercariae were counted and picked with a pipette mounted onto a rubber bulb and dispensed into 5ml beakers. Mice were percutaneously infected with 200 cercariae/animal following a procedure adapted from the ring method (Tendler & Pinto, 1981). Mice were anesthetized with 50µl of ketamine (100mg/ml; Agrar Holland BV, Soest, The Netherlands). After shaving the abdomen, an infection ring was attached on the abdomen into which cercariae suspension was then poured and left for 30 minutes to penetrate the mice skin. The mice were then maintained in the IPR rodents’ house in groups of five for seven weeks.
2.7 Parasitological assays

2.7.1 Faecal eggs recovery through Kato katz method

Faecal schistosome eggs count was done on the 5th, 6th, and 7th, week post challenge in duplicates per mice using Katz et al., (1972) method. Mice were transferred into individual cages with no beddings in each of these tests where the 24 hour faecal material was collected, weighed and processed for egg counting using Kato Katz technique. Coverslips to be used were soaked in 50% glycerine malachite green solution 24 hours before use. After mixing the stool sample, about 1-2 grams of faecal material was forced through a 125µm mesh and the stool recovered at the back of the mesh. Using a template, a 50mg faecal specimen was transferred to a microscope slide and then covered with the cellophane coverslip soaked in the 50% glycerine malachite green solution. Slides were kept 24 hours in the dark then observed under the microscope at ×10 magnification. Eggs per gram were obtained by multiplying the average number of eggs per slide by 20.

2.7.2 Perfusion method

Infected mice were perfused 7 weeks post challenge to recover adult worms using a modified method of Smithers and Terry (1965). Each mouse was anaesthetized by injecting it with 100 µl of heparinized ketamine (1 ml of heparin into 5 ml of ketamine). A transverse mid-ventral cut was made on the skin of the abdomen and the mouse skin peeled round the ‘waist’ of the animal. Thoracic cavity and the abdomen wall were then opened up without cutting the viscera. Ribs on either side of the sternum were snipped off and the liver observed for any gross pathology. The hepatic portal vein was then located, incised and the area flushed with perfusion fluid (0.85% sodium chloride and 1.5%...
sodium citrate) to remove any worms and prevent clotting. A 21 gauge needle connected to the tube with perfusion fluid was inserted into the left ventricle and perfusion fluid pumped through until the liver became pale in color and the mesenteric veins were clean. Recovered (normal and stunted) male and female worms were counted separately and the average found per each group. A male worm of between 6-13 mm in length and 0.8-1.0 mm in width and a female worm of 7-17 mm in length and 0.25 mm in width were considered normal while those worms that were smaller than this were classified as stunted (Loker, 1983).

2.7.3 Adult worm recovery

Adult worms were recovered using the method of Yole et al. (1996) where the perfusate containing the recovered worms in urine glass jars was topped up with phosphate buffered saline (PBS) and haemolyser DL-1 (Erma Inc. Japan) added to lyse the red blood cells. The supernatant was carefully sucked out after the worms had settled down and the washing procedure repeated three times. The recovered worms were then placed on a Petri dish with PBS and counted under a dissecting microscope. Resistance to challenge infection was expressed as the percentage reduction in the number of adult worms recovered from mice receiving hyper-immune serum or infection serum compared with non immunized control mice calculated as follows:

\[
\text{Resistance} = \frac{(C-E)}{C} \times 100
\]

Where C represents the mean recovery from challenge control mice and E represents the mean recovery from experimental mice (mice receiving hyper-immune serum or infection serum).
2.7.4 Tissue egg count

Frozen liver samples collected 7 weeks post infection during perfusion were finely chopped and put in 100ml vortex beaker with (20-30) ml of PBS and processed for eggs recovery using the method adapted from Liu et al., (1995). Samples were blended for 3-4 minutes and the emulsion transferred to conical flasks with 5% volume of 5% potassium hydroxide (KOH). Samples were then left overnight at room temperature and at 45 °C for 3 hours. Sample volume was then recorded and eggs randomized by bubbling air through. A counting slide was filled with randomized egg suspension in both chambers and eggs lying in the 24 grid squares counted with a dissecting microscope at × 40 magnifications. Number (N) of eggs in the original organ was calculated as follows;

\[ N = \frac{W}{w} \times V \times 24/n \times 1.05(r_1+r_2)/2 \]

Where;

- W and w = the weights in grams of the whole organ and the sub sample respectively.
- V = the volume in ml of the digest after incubation.
- n = the number of grid squares counted.
- \( r_1 + r_2 \) = the number of eggs counted in each of the two chambers.

2.8 Bleeding of mice for serum

Blood samples were collected from tail veins of all mice prior to immunization in experiment two and thereafter at 2, 4 weeks interval and finally at 7 weeks post challenge during perfusion through heart puncture. For experiment one, blood samples were obtained during perfusion through heart puncture. Pooled serum samples for ELISA were prepared from each group by mixing an equal volume of serum from each group.
2.9 Pathological examination

2.9.1 Gross pathology

Gross pathological examination of the liver focused on its general appearance. The observations considered in the liver included the color, size and the presence of granulomas. The presence or absence of adhesions was also considered. An arbitrary score of 0 to 4 was used to categorize gross pathology (Dragana et al., 1998). A score of 0, 1, 2, 3, 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe pathology respectively.

2.9.2 Histopathology

Liver sections from sacrificed mice were collected 7 weeks post-infection during perfusion and fixed in 10% buffered formalin for two weeks according to the method of Bancroft and Stevens, (1982). Optimum dehydration was achieved by immersing the samples into 80%, 95% and 100% ethanol respectively. The tissues were then cleared in toluene and infiltrated in hot paraffin. They were embedded on tissue-embedding paraffin wax (Sherwood Medical co. USA) and then sectioned serially at 6 microns using a rotary microtome (Leitz, Germany). The tissue sections were mounted on glass slides and stained (Appendix I) with haematoxylin and eosin (H and E). Using a calibrated ocular micrometer, the tissue sections were observed under a light microscope at 100 × magnification and the number and size of granulomas recorded. Granuloma size was measured based on the vertical and horizontal diameters of granulomas with a visible centrally placed schistosome egg (Farah et al., 2000). The horizontal and vertical diameter average was taken as the diameter of the granulomas.
2.10 Immunological assay; Enzyme-linked immunosorbent assay (ELISA)

Soluble egg antigen (SEA) and Soluble adult worm preparation (SWAP) specific IgG responses in mice after challenge infection were determined by titration of the serum in different concentrations of these antigens. The ELISA technique employed is a modification of Hillyer and Gomez (1979) method. Coating concentrations of the antigens and the sera dilutions factor were determined after the titration of the serum against different concentrations of the antigens. The antigens were used at coating concentrations of 2µg/ml for SEA and 5µg/ml for SWAP while the serum was diluted as follows; SEA (1:400) and SWAP (1:200). The antigens were diluted with 1× phosphate buffered solution (1× PBS) from a stock of 1.4 mg/ml for SWAP and 1 mg/ml for SEA while serum was diluted in 1× PBS- Tween (0.05%). 96 well immunosorp ELISA plates (Dynex) were coated with the diluted antigen, 50 µl/well, and the plates incubated at 4°C overnight. The plates were then washed three times with PBS-Tween (0.05%).

Non-specific binding sites were blocked by adding PBS-Tween (0.5%) 250 µl/well and then incubated for 2 hours at 37°C. 50 µl of the diluted serum was added into each well and the plates incubated overnight at 4°C. The plates were then washed six times with wash buffer and 50 µl of diluted anti-mouse IgG conjugated to HorseRadish Peroxidase (Bio-Rad Laboratory) added to each well at a working dilution of 1: 2000 in 1 × PBS-Tween (0.05%). The plates were then incubated for 1 hour at 37°C and then washed 6 times. Detection was done by the addition of 50 µl of 3,3’, 5,5’-tetra methylbenzidine (TMB microwell peroxidase substrate solution, Kirkegaard and Perry Labs, Gaithersburg,
Maryland, USA) into the wells. After a further 25 minutes incubation, absorbance was measured as optical density (OD) at 630 nm using an ELISA reader (Dynatech MR500).

2.11 Data analysis

Statistical analysis was done using Statistica software and Microsoft excel. Data were characterized by their means and standard error (mean ± Standard error). Analysis of variance (ANOVA) was used to compare the difference between means of variables. The $p$-values are one-tailed ( $p > 0.05$ is not significant, $p < 0.05$ is significant). Resistance to challenge infection was expressed as the percentage reduction in the number of adult worms recovered from mice receiving hyper-immune serum or infection serum compared with non immunized control mice calculated as follows:

$$\text{Resistance} = \frac{C-E}{C} \times 100$$

Where C represents the mean recovery from challenge control mice and E represents the mean recovery from experimental mice receiving hyper-immune serum or infection serum (Mangold and Dean, 1986).
3. 1 Determination of the role of antibodies against the pre-liver-lung stage schistosomulae

Effect of immune serum generated from RA vaccinated baboons on the pre-liver-lung stage schistosomulae was determined by comparing the number and nature of schistosome worms recovered, number of faecal and trapped schistosome eggs in the liver and on the size and number of granulomas in the liver. The levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) were also compared in all the groups.

3.1.1 Effect of Immune serum from RA vaccinated baboons on the number and nature of schistosome worms.

The percentage worm reduction in this study is shown in Table 3.1. The mean number of S. mansoni worms recovered from mice immunized with hyper-immune serum, those immunized with infection serum and from the control was 49, 47.2 and 44.6 respectively. The difference in mean worm burdens between each of the immunized groups and that of control was however not statistically significant (P > 0.05). The table shows that mice receiving hyper-immune serum recorded the highest number of worms as compared to the control and infection serum recipients. In this study there were more male worms recovered compared to the female worms in all the groups after perfusion. Compared to the females, there were more stunted males in all the groups (Table 3.2). The difference in mean stunted worm between each of the immunized groups and that of control was statistically not significant.
Table 3.1: Mean Number of worms after passive immunization on the 7th and 14th day post-challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Passive transfer</th>
<th>Worm recovery (mean ± S.E)</th>
<th>% Protection</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-immune serum recipients</td>
<td>With hyper-immune serum</td>
<td>49 ± 9.7</td>
<td>-9.9</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>With infection serum</td>
<td>47.2 ± 10.8</td>
<td>-5.8</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>Without serum</td>
<td>44.6 ± 9.7</td>
<td>-</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 3.2: Normal and stunted schistosome worms recovered after passively immunizing mice on the 7th and 14th day post challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Female worms</th>
<th></th>
<th>Male worms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Stunted</td>
<td>Total</td>
<td>Normal</td>
</tr>
<tr>
<td>Hyper-immune serum recipients</td>
<td>83</td>
<td>1</td>
<td>84</td>
<td>151</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>90</td>
<td>0</td>
<td>90</td>
<td>141</td>
</tr>
<tr>
<td>Control</td>
<td>75</td>
<td>1</td>
<td>76</td>
<td>143</td>
</tr>
</tbody>
</table>
3.1.2 Effect of Immune serum from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver

The effect of hyper-immune serum from baboons vaccinated with RA vaccine against the pre-liver-lung stage schistosomulae was first determined by comparing the average number of 24 hour faecal eggs at weeks 5, 6 and 7 from both the immunized and the controls. There were no eggs detected in all the groups at week 5 and 6. At week 7, mice immunized with hyper-immune serum had the lowest mean number of eggs, (42.6) followed by mice immunized with infection serum with a mean of 74.8. The control group had the highest egg count with a mean of 86 though the differences between the means were not significantly different (p > 0.05, Fig. 3.1).
Figure 3.1: Mean number of schistosome faecal eggs (24 hour) in mice immunized on the 7th day and boosted on day 14 post challenge and the control group (means + SE).
The effect of immune serum from RA vaccinated baboons on the number of trapped schistosome eggs in the liver was also evaluated. There was a reduction in the mean number of eggs in the liver tissue of mice receiving hyper-immune serum and infection serum. The control group had the highest number of liver eggs while the mice that received infection serum had the lowest though the difference between the immunized groups and the control was not statistically significant (P > 0.05, Figure 3.2).

Figure 3.2: Mean number of liver schistosome eggs in mice immunized on the 7th day and boosted on day 14th post challenge (means + SE).
3.1.3 Effect of Immune serum from RA vaccinated baboons on the size and number of liver granulomas

The pathological findings in this study were divided into gross pathology and histopathology. Gross pathological examination of the liver showed that granulomas formed in all mice at week seven. Livers from both the immunized and the control had a whitish/creamy spots on the surface. Livers from all animals appeared pale compared to the red pink hue in the normal mice although the size of the liver lobes was not affected. As stated earlier on (Dragana et al., 1998), a score of 0 to 4 was used to categorize gross pathology. A score of 0, 1, 2, 3, 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe gross pathology respectively.

In the group immunized with hyper-immune serum, two mice had severe granulomas while the other three had very severe granulomas. In the group immunized with infection serum, only two mice had severe pathology while the other three had very severe granulomas. The most severe pathology was recorded in the non immunized group where four mice with very severe granulomas and one mouse with between severe to very-severe granulomas were observed. Adhesions were observed in all mice except one from the control group.

Scores of liver granulomas is as shown in Table 3.3 below. The mean granuloma size was calculated from the average measurement of the vertical and horizontal diameters of granulomas in each slide. Among the three different groups, there was no significant difference in the size of the granulomas. Mice immunized with the protective serum recorded the highest average size of granulomas while the lowest size was observed in
the control group though this difference was not statistically significant (P > 0.05). Mice receiving hyper-immune serum recorded the lowest number of granulomas as compared to the rest of the groups though not statistically significant (P > 0.05, Table 3.3). There was thus no significant difference between granuloma number and size in mice that received hyper-immune serum, infection serum and the control mice that were not immunized. Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining) in this experiment is as shown below in Figure 3.3.

Table 3.3: Number and size of liver granulomas after passive immunization on the 7th and 14th day post challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Granuloma Number (mean ± S.E)</th>
<th>P-value</th>
<th>Granuloma Size (µm) (mean ± S.E)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-immune serum recipients</td>
<td>11 ± 1.55</td>
<td>&gt; 0.05</td>
<td>37.56 ± 1.45</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>18 ± 3.01</td>
<td>&gt; 0.05</td>
<td>36.27 ± 0.81</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 3.81</td>
<td>&gt; 0.05</td>
<td>34.34 ± 1.69</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
3.1.4 Levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP)

The challenge infection with *S. mansoni* stimulated IgG antibody responses in C57Bl/6 mice. SWAP and SEA antigens were used to probe perfusion sera in the three groups of mice. Mice immunized with hyperimmune serum had the lowest response for both SWAP and SEA. The highest response to SWAP and SEA was recorded in mice transfused with infection serum and the control groups respectively (Figure 3.4). The differences in the means were significantly different for the IgG response to SWAP (P < 0.05) though not significantly different (P > 0.05) for the response to SEA.

Figure 3.3: Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining). A (Hyper-immune serum recipients), B (Infection serum recipients), C (Control)
Figure 3.4: IgG response to SWAP and SEA at perfusion in mice immunized on the 7th day and boosted on day 14 post challenge.
3. 2 Determination of the role of antibodies against the migrating larvae

Effect of immune serum generated from RA vaccinated baboons on the migrating larvae was determined by comparing the number and nature of schistosome worms recovered, number of faecal and trapped schistosome eggs in the liver and on the size and number of granulomas in the liver. The levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) were also compared in all the groups.

3.2.1 Effect of Immune serum from RA vaccinated baboons on the number and nature of schistosome worms

Male and female worms recovered through portal perfusion at week 7 were counted and the results expressed as mean total worms (male + female). The percentage worm reduction in this study is as shown in Table 3.4.

Table 3.4: Worm recovery after passive immunization on the 3\textsuperscript{rd} day post challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Passive transfer</th>
<th>Worm recovery (mean ± S.E)</th>
<th>% Protection</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-immune serum recipients</td>
<td>With hyper-immune serum</td>
<td>43.8 ± 8.6</td>
<td>25.8</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>With infection serum</td>
<td>71 ± 20.8</td>
<td>-20.3</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>Without serum</td>
<td>59 ± 14.5</td>
<td>-</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
The mean number of *S. mansoni* worms recovered were 43.8, 71 and 59 from the hyper-immune serum recipients, infection serum recipient and control, respectively (Table 3.4). This shows that mice receiving hyper-immune serum recorded the lowest number of worms as compared to the control and infection serum recipients. This translated into 25.8% protection in the hyper-immune serum recipient while mice receiving infection serum were not protected. The difference in mean worm burdens between each of the immunized groups and that of control was statistically not significant (P > 0.05). There were more total male worms recovered compared to the total female worms in all the groups at perfusion. In this experiment also, there were more stunted males in all the groups as compared to the females (Table 3.5). The difference in mean stunted worms between each of the immunized groups and that of control was statistically not significant.

**Table 3.5: Normal and stunted schistosome worms recovered after passively immunizing mice on the 3rd day post challenge**

<table>
<thead>
<tr>
<th>Group</th>
<th>Female worms</th>
<th>Male worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>stunted</td>
</tr>
<tr>
<td>Hyper-immune serum recipients</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>122</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>94</td>
<td>2</td>
</tr>
</tbody>
</table>
3.2.2 Effect of Immune serum from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver

The efficacy of hyper-immune serum from baboons vaccinated with RA vaccine against the migrating *Schistosoma mansoni* was determined first by comparing the average number of 24 hour faecal eggs at weeks 5, 6 and 7 from both the immunized and the controls (Figure 3.5).

![Figure 3.5: Mean number of schistosome faecal eggs in mice immunized on the 3rd day post challenge (means + SE).](image-url)
There were no eggs detected in all the groups at week 5. Eggs were detected only in the hyper-immune serum recipient and the control group at week 6. At week 7, mice immunized with hyper-immune serum had the highest mean number of 24 hour egg count, 130.4 followed by the control group with a mean of 110.6. Mice immunized with infection serum had the lowest count with a mean of 60 though the differences between the means were not statistically significant ($P > 0.05$, Fig. 3.5).

The effect of immune serum from RA vaccinated baboons on the number of trapped schistosome eggs in the liver was also evaluated in this experiment. Mice receiving hyper-immune serum had the lowest egg count while the highest count was recorded in the control group. The differences between the group means were however not statistically significant ($P > 0.05$, Figure 3.6).
3.2.3 Effect of Immune serum from RA vaccinated baboons on the size and number of granulomas

Both the gross and the histopathological examination of the livers was carried out on the mice immunized on the 3rd day after infection. A score of 0 to 4 was used to categorize gross pathology (Dragana et al., 1998). A score of 0, 1, 2, 3, and 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe gross pathology, respectively. Gross pathological examination of mice liver immunized on the
third day after challenge infection showed that granuloma had formed in all groups of mice except one mouse in the infection serum recipients group. In the hyper-immune serum recipient group, three mice had severe granulomas while the other two mice had very severe and moderate-severe granulomas, respectively.

Though there were no observed granulomas in one mouse in the infection serum recipient group, two mice from this group had very severe granulomas while the other two had between moderately-severe granulomas. In the control group, one mouse had very few granulomas, two mice had severe granulomas and one mouse recorded an average of moderate to severe granulomas. In the control group, only one mouse had very severe granulomas. Liver adhesions were detected in three, four, and three mice in the hyper-immune serum recipients, infection serum recipients, and control group, respectively. It was only in the infection serum recipients group that one mouse had both the liver and lung adhesions.

Histopathological examination of the liver in the three different groups showed no significant difference in the size and number of the granulomas. Mice immunized with the infection serum recorded the lowest average number of granulomas while the control group had the largest average though this difference was statistically not significant ($P > 0.05$). The same was observed in the size of granulomas where mice immunized with infection serum had the lowest average size and control group recorded the highest average with no significant differences. There were no granulomas observed in one mice in the infection serum recipients group and this translates to a large standard error
recorded in this group especially in the size of granulomas (Table 3.6). Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining) in this experiment is as shown below in Figure 3.7.

Table 3.6 Number and size of liver granulomas after passive immunization on the 3rd day post challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Granuloma Number (mean ± S.E)</th>
<th>P-value</th>
<th>Granuloma Size (µm) (mean ± S.E)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-immune serum recipients</td>
<td>18 ± 4.33</td>
<td>&gt; 0.05</td>
<td>38.46 ± 1.72</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Infection serum recipients</td>
<td>15 ± 5.18</td>
<td>&gt; 0.05</td>
<td>27.14 ± 6.87</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>20 ± 5.87</td>
<td>&gt; 0.05</td>
<td>41.95 ± 3.23</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Figure 3.7: Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining). A (Hyper-immune serum recipients), B (Infection serum recipients), C (Control) in mice immunized on the 3rd day post challenge.
3.2.4 Levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP).

To establish whether IgG antibody production differed significantly between the three groups, antibody responses to SWAP and SEA were measured at 0 day before challenge, 2 weeks after challenge, 4 weeks after challenge and at perfusion (7 weeks after challenge). The mean levels of IgG responses is shown in Figure 3.8 and 3.9 below for SWAP and SEA respectively.

The first two weeks were marked with a low or nearly no detectable response followed by a gradual increase in IgG antibodies for both SWAP and SEA antibodies throughout the infection period. This response to both SWAP and SEA was observed in all the groups at all time points without a significant difference between the groups at any of these assayed points. Mice immunized with infection serum recorded the lowest response in both assays but the observed difference as compared to the rest of the groups was not statistically significant. Immunization with either the hyper-immune serum or the infection serum did not affect the animals response to both SWAP and SEA as the observed gradual increase was recorded in all the groups.
Figure 3.8: IgG response to SWAP in mice immunized on the 3rd day post challenge.
Figure 3.9: IgG response to SEA in mice immunized on the 3rd day post challenge.
4.1 DISCUSSION

The role of humoral response induced after RA vaccination was studied by passively immunizing mice with either hyper-immune baboon serum from RA vaccinated baboons or with baboons infection serum. The results of this study demonstrate that passive immunization of mice with 500µl of hyper-immune serum from baboons vaccinated with RA vaccine did not provide significant protection against Schistosoma mansoni in C57Bl/6 mice contrary to the findings previously reported in the literature (Mangold and Dean, 1986; 1992). This lack of significant protection was noted irrespective of the timing of serum injection after percutaneous infection. In both studies, no eggs were detected in all the groups on the 5th week after infection. Eggs were detected only in one mice immunized with hyper-immune serum and from one mice in the control at week 6 in the mice immunized on the 7th day and boosted on the 14th day post challenge. Similarly, there were no eggs detected at week 6 in the mice immunized on the 3rd day post-challenge and this is probably due to the low sensitivity of kato katz technique (Kongs et al., 2008). The two experiments performed were of similar design varying only in the days of passive immunization.

Passive transfer of immunity by humoral factors has previously been studied by several investigators (Cook et al., 1972; Bickle et al., 1985; Mangold and Dean, 1992). In the present study where the role of humoral immunity against migrating schistosome was
evaluated by passively immunizing mice, a 25.8 % protection level was obtained though the mean worm burden in the immunized group compared to the control was not significant. This could suggest that the transferred hyper-immune serum could be involved in protection. There was no protection obtained when mice were immunized on the 7th day and boosted on the 14th day probably suggesting that the immune effector mechanism in this hyper-immune serum targets the migrating schistosomulae and not the pre-liver-lung stage schistosomulae. This is supported by findings of other workers who showed that the newly transformed schistosomulae is the most likely target of antibody mediated attack (Sher et al., 1984).

Heterologous transfer of immune serum from rats and rabbits to mice has been shown to afford significant protection in the recipients thus indicating that similar mechanisms of parasite elimination may be occurring in these species (Mangold and Dean, 1992; Ford et al., 1984). In this study, the diverging results between two similar immunization protocols could perhaps be explained by the use of serum from animals of greater genetic and physiologic difference to the serum recipients. The failure to transfer protection by passive immunization with the hyper-immune serum may also suggest that antibodies generated after vaccination with RA vaccine in baboons may not play a key role in mediating protection in mice. Failure to transfer resistance to mice with antibodies after vaccination has also been noted by Verity et al., (2001) who evaluated the efficacy of recombinant cathepsin D spartic protease from *Schistosoma japonicum.*
Sher et al. (1982) demonstrated that nude mice and mice treated from birth with anti-μ chain serum failed to develop anti-schistosomular surface antibodies or even manifest resistance following vaccination with irradiated cercariae is an indication that the humoral immunity is involved in this type of acquired resistance. Studies on species specificity of immunization and attempts to transfer resistance by Bickle et al. (1985) showed that though significant levels of resistance against Schistosoma mansoni challenge were developed in mice exposed to highly irradiated (20 krd.) cercariae of the homologous species (53-67%), their attempt to transfer this resistance to naive recipients by injection of serum and or spleen or lymph node cells from the vaccinated donor mice were largely unsuccessful. In contrast, significant levels of resistance have been transferred to mice by injection with serum from rabbits exposed to irradiated cercariae (20 krad.). This protection translated to 34-69% when serum was injected at the time of challenge or 31-56% when injected 5-6 days later (Bickle et al., 1985).

These findings that sera from rabbits vaccinated with 20 krad. can confer significant protection to mice suggest that specific serum-borne factors stimulated by irradiated infections can kill schistosomula in vivo in mice. This inability of hyper-immune serum from baboons to transfer significant protection in this study could probably be due to differences in titre, isotype or specificity of the antibody responsible for protection (Verity et al., 2001). In the mice immunized on the 3rd day post challenge, one mice in the infection serum recipient group had only two worms that were recovered and this could account for the large standard error recorded in this group.
There was no protection achieved when mice were immunized with infection serum in the two studies. Infection serum used in this study coincided with egg production from the serum donors. It has been previously reported that schistosomes eggs stimulate the production of antibodies that are reactive with the antigens on the surface of skin-transformed schistosomulae but which fail to offer any significant protection (Ford et al., 1984). Early studies on homologues immune serum ware met with limited success thus making the understanding of the mechanisms underlying resistance to Schistosoma mansoni infection in either man or laboratory hosts to be more difficult. In humans, transfer of anti-schistosome gamma globulin to children living in an endemic area failed to protect a significant proportion of the recipients from contracting the disease (Cook et al., 1972). Also an attempt to transfer homologous immune serum to rhesus monkeys failed to establish significant protection in recipients (Ogilive et al., 1966).

The time of serum injection appeared to be crucial in conferring protection against challenge infection as has also been shown by other researchers (Jwo and Loverde, 1989; Mangold and dean, 1992). Studies have shown that comparable levels of resistance could be transferred to mice with rabbit serum given at the time of challenge or 5-7 days later thus demonstrating that the challenge schistosomula remain susceptible up to or beyond the lung stage (Bickle et al., 1985). This is consistent with the results of experiment one since a 25.8% protection level was obtained when mice were immunized on the 3rd day. The site of parasite immune elimination still remain unclear since passive immunization on the 7th day and boosting on the 14th day post challenge failed to confer any significant protection (Coulson and Mountford, 1989; Richter et al., 1995).
Granuloma formation has been defined as the inflammatory response against parasite eggs that are trapped in host tissues. The intensity of this response is either modulated by the host (Goes et al., 1991) or by the parasite factors (Boulanger et al., 1992). Anti-schistosome monoclonal antibodies have been shown to confer resistance against challenge infection with *Schistosoma mansoni* resulting in a significant decrease in granuloma number and size (Attallah et al., 1999). This reduction in the size and number of granulomas was found by Attallah et al. (1999) to be consistent with an effective reduction in the number of schistosome worms *in vivo*.

For a reduction in the granuloma number to be experienced, it would be expected that the specific antigen would induce an immune response in the host which would then cause a severe damage to the essential life functions of the parasite leading to its death and consequently a reduction in the number of eggs produced (Grzych et al., 1993). This protection is provided during a passive immunization and if the target antigen in the developing worms are present, then a reduction in the number of worms and the eventual reduction in the number of egg granulomas would be expected. Lack of an eventual significant reduction in the number of worms in the immunized mice as compared to the control could thus account for the recorded high liver eggs in all the groups and the eventual lack of significant difference in the number and size of granulomas between the immunized and the controls (Attallah et al., 1999).

There were no granulomas observed in one mice in the group immunized with infection serum on the 3rd day and this could probably account for the low averages recorded in
number and size of granulomas in this group. This thus translates to a large standard error recorded in mice immunized with infection serum on the 3rd day especially in the size of granulomas (Table 3.6). This is consistent with the parasitological findings in this experiment as there were no eggs detected in the faecal matter or in the liver of this mouse.

There could be several reasons why baboon serum containing a high activity of anti-schistosomular antibody failed to protect the mice. It is possible that the humoral components from baboon may not be able to cooperate with the mouse effector system necessary in killing the schistosomulae. This is supported by findings of Lewis et al., (1977) who observed that plasma from humans infected with Schistosoma mansoni failed to protect mice from Schistosoma mansoni cercariae challenge. RA vaccine experiments in mice have shown that the secretion of abundant interferon-γ (IFN-γ) as part of a cell-mediated response in the lungs is crucial to the establishment of optimum levels (60–70%) of protective immunity (Coulson, 1997). Anti-parasite antibodies generated in mice after single vaccination with RA vaccine are thought not to contribute towards protection since antibody titers in different strains of vaccinated mice do not correlate with the level of immunity, neither does serum transfer to naive recipients confer passive protection (Richter and Harn, 1993; Coulson, 1997). It is thus possible that the mechanisms of protection in mice do not totally rely on humoral components a phenomenon that is yet to be proved.
4.2 CONCLUSIONS

The following conclusions can be made from the results of this study;

i. There was no effect of hyper-immune serum from RA vaccinated baboons on the number and nature of schistosome worms in C57Bl/6 mice.

ii. Passive immunization of C57Bl/6 mice with hyper-immune serum from RA vaccinated baboons did not affect the fecundity of schistosome worms irrespective of the timing of serum injection since there was no significant difference in the number of faecal and trapped schistosome eggs in the liver of all groups.

iii. There was no significant reduction of liver granulomas in both size and number after passive immunization irrespective of the timing of serum injection.

iv. There was no significant protection that was conferred in mice against pre-liver-lung stage schistosomulae and or the migrating schistosome larvae by hyper-immune serum from RA vaccinated baboons probably due to the inability of the baboon serum to cooperate with the mouse effector system necessary in killing the schistosomulae.

Therefore, the null hypotheses which stated that:

Ho$_1$: Hyper-immune serum from baboons does not protect mice against pre-liver-lung stage schistosomulae was accepted and the alternate hypotheses rejected.

Ho$_2$: Hyper-immune serum from baboons does not protect mice against the migrating schistosome larvae was accepted and the alternate hypothesis rejected.
4.3 RECOMMENDATIONS

i. Homologous passive transfer of hyper-immune serum (baboon to baboon) should be experimented on.

ii. Other stages of the schistosome lifecycle should also be targeted during passive transfer experiments.

iii. The mechanisms of protection in mice should be further evaluated.
REFERENCES


Schistosoma mansoni antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunology*, **17**: 297–304.


### APPENDIX I

**HARRIS’ HEAMATOXYLIN STAIN**

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<th>Ingredient</th>
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<td>Hamatoxylin crystals</td>
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<td>Ammonium or potassium alum</td>
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**EOSIN STAIN**

**Eosin Stock Solution**

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**Eosin Working Solution** *(used to stain slides)*

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